

REMARKS

1. Based on the Office Communication, the applicants have further amended the claim 1 to clarify the difference between this application and the combination of Caplan et al., Rieser et al and Butz et al prior arts by changing the preamble to be isolating but not recovering. This application has further added the limitation of providing a cell mixture in a culture medium [0030], the limitation of seeding and culturing the cell mixture [0030 and 0035], and the upper plate to be made of the mesenchymal stem cell adhering material [0029 and 0030]. The plastic material was mentioned in [0007] section of this application. The culturing medium was disclosed in [0030] section of this application.
2. The method taught by Caplan et al does not provide the bone marrow aspirate with a culture medium that facilitates mesenchymal stem cell growth at the same time. Further, this application has provided a method for isolating, culturing and expanding mesenchymal stem cells to confluence at the same time. Therefore, it would not be obvious to one of ordinary skill in the art to modify the method of isolating mesenchymal stem cells of Caplan et al.
3. The obvious rejection was made to combine the method of isolating mesenchymal stem cells of Caplan et al and the culturing dish taught by Rieser et al. The combination would be to use a filter to remove red blood cells from bone marrow aspirate and Rieser et al. the use of a porous filter. As the Office Action indicated, Caplan et al teach a method of recovering mesenchymal stem cells from bone marrow, comprising the steps of (i) providing the bone marrow aspirate, (ii) seeding the cell mixture in a device, and (iii) recovering the mesenchymal stem cells from filter. As mentioned above, the method taught by Caplan et al does not provide the bone marrow aspirate with a culture medium at the same time. Therefore, further modification to use the porous filter taught by Rieser et al is still quietly different from the whole process of this application, which comprising (a) providing a cell mixture comprising mesenchymal stem cells and other cells in a culture medium, said the culturing medium containing factors that stimulate mesenchymal stem cells growth without differentiation and allowing for the selective adherence of only the mesenchymal stem cells to substrate surface; (b) seeding and culturing the cell mixture in a culture device comprising an upper plate with pores and a lower plate base, and (c) removing non-adherent cells on the upper plate by changing medium. **The steps removing non-adherent cells of this application are not identified in the prior arts.**

Furthermore, as the Office Action indicated, Rieser et al teach that bone substitute plate (7) serves two functions: it is a permeable wall for the cell space (1), and it provide a substrate for adherence of cells. Moreover the abstract of Rieser et al clearly indicated that “The cells settle on such a plate (7) and the cartilage tissue growing in the cell space (1) **grows into pores or surface roughness of the plate**, whereby an implant forms which consists of a bone substitute plate (7) and a cartilage layer covering the plate and whereby the two implant parts are connected to each other in positively engaged manner by being grown together.” However, the pores of this application do not function for cell growth, but **the pores are used to separate the mesenchymal stem cells from other cells through the pores.**

Moreover, since the **bone substitute plate taught by Rieser et al is used for implantation**, it would be not obvious to removing non-adherent cells by changing medium to isolate the mesenchymal stem cells from the upper plate as indicated in this application. For implantation purpose, one would expect to rich the bone substitute plate as much cells as possible. **How could one think to remove non-adherent cells from plate of implantation?** Actually, it would be also difficult to removing addition cells and recovering mesenchymal stem cells from the bone substitute plate because of surface roughness. Not only the US patent 5634879 clearly stated that “It is believed that this roughened surface exposes a greater surface anchoring area to cells for attachment.” (column 6, lines 2-4), but also the **claim 14 of Rieser et al includes mesenchymal stem cells to grow into pores or surface roughness of the plate.**

Other than the difference in steps, the function, way and result of the upper plate in this application are also different from the combination of Caplan et al and Rieser et al.

| | This application | Caplan et al + Rieser et al |
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| function | The pores of the upper plate is to separate the mesenchymal stem cells from other cells through the pores | growing in the cell space grows into pores or surface roughness of the plate |
| way | the upper plate made of the mesenchymal stem cell adhering material, said plastic | bone substitute plate is rigid. , plastically deferrable, biologically degradable or not degradable. |
| result | isolate mesenchymal stem cells from the bone marrow aspirate | to implant the bone substitute plate |

4. As we mentioned before, Rieser et al suggested that “it is not necessary to isolate specific cell types from donor tissue, i.e. mixtures of different cells as usually contained in such tissues can be brought into the cell space as such.” (column 5 lines 26-28) It would be not obvious to one of ordinary skill in the art to combine Rieser et al with the isolating method of Caplan et al. Furthermore, Rieser et al discredited the filter material in US Pat. No 5326357. (column 2 lines 42-46) Therefore, one of ordinary skill in the art would be very possible not to use the bone substitute plate (7) of Rieser et al as a filter. Moreover, how would the one of ordinary skill in the art think the bone substitute for implantation to be used a filter?

Actually, Rieser et al also claim mesenchymal stem cells to grow into pores or surface roughness of the plate (claim 14). Therefore, there is a difficulty to recovery mesenchymal stem cells from the bone substitute of Rieser et al. Even though the cell space taught by Rieser et al. functions as a filter, the result of isolating efficiency is still far behind from this application because that the surface roughness of bone substitute exposes a greater surface anchoring area to cells for attachment. Therefore, it would not be obvious to one of ordinary skill in the art at the time this invention was made to modify the methods of Caplan et al and Rieser et al.

5. The new rejection over Caplan et al in view of Butz et al is to use Leukosorb™ filter of Caplan et al for the separation of human mesenchymal stem cells from fat cells and red blood cells from bone marrow aspirate and Butz et al teach culturing cells in a device comprising a porous filter. Again, **other than the difference in steps** as mentioned above, the function, way and result of the upper plate in this application are also different from the combination of Caplan et al and Butz et al.

| | This application | Caplan et al + Butz et al |
|----------|---|--|
| function | The pores of the upper plate is to separate the mesenchymal stem cells from other cells through the pores | The cell retention element includes a porous membrane growth surface.(column 1 lines 56-57) |
| way | the upper plate made of the mesenchymal stem cell adhering material, said plastic | It is formed of two separate pieces, rather than as a unitary piece as in the transwell. (column 1 lines 44-45) The retention element 10 has a hollow, cylindrical side wall defining an annular bottom surface. Attached to the annual surface is a tissue growth member. The tissue |

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| | | growth membrane may be formed of any material capable of supporting cells or tissue substantially isolated from direct contact with medium in the wall, while allowing at least selected material to pass through and contact the cells. Such materials include porous iner film,...(column 6 lines 14-22) |
| result | isolate mesenchymal stem cells from the bone marrow aspirate | A tissue or cell growth device |

In sum, not only the steps of this application are different from the prior arts, but also the function, way and result of the pores of the upper plate in this application are distinct from the combination of the prior arts. Accordingly, this application should be placed in condition of allowance. An early Notice to this effect is respectfully expected.

Respectfully submitted:

By: Shih-Chieh Hung

Shih-Chieh Hung

886-2-28757557 ext 118 (voice)

886-2-28265164 (fax)

hungsc@vghtpe.gov.tw(email)

No 201 Shih-Pai Rd Section II,
Taipei-Veterans General Hospital, Taipei,
Taiwan, R. O. C.